Quality Changes of Cucumber Grafted Transplants as Affected by Long-term Exposure to Continuous Darkness and Low Temperature Conditions

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ABSTRACT

This study was conducted to investigate deterioration of cucumber grafted transplant during storage under continuous darkness and low temperature conditions. In the first chapter of this thesis, after cucumber grafted transplants were stored at 6, 9, 12, 15, and 18^{o}C for 3, 6, 9, 12, and 15 days in continuous darkness, stem elongation and chlorophyll degradation of cucumber grafted transplants during storage were suppressed as storage temperature reduced.

Chlorophyll content of stored cucumber grafted transplants was significantly reduced with increasing storage air temperature. The regrowth of stored transplants in 15 days after transplanting, the vigor of cucumber grafted transplants stored for 3 days was not suppressed. As storage period prolonged, however, the storage conditions affected negatively the vigor of cucumber grafted transplants after transplanting. At 6^{o}C, cucumber grafted transplants showed the lowest stem elongation and chlorophyll degradation among all treatments during 15 days of storage and showed no sign of vigor loss after transplanting until 3 days of storage. In the second chapter, cucumber grafted transplants were stored under constant or increasing air temperature conditions in continuous darkness. The hypothesis of this chapter was that the cucumber grafted transplant storage at 6^{o}C for first 3 days and following storage at increasing air temperatures may reduce chilling stress caused by low temperature and induce self-recovery of cucumber grafted transplants from physiological damages occurred during storage. Cucumber grafted transplant stored at 6^{o}C first 3 days showed no more stem elongation even though the subsequent storage temperature increased to some extent. In addition, changes in stem elongation, chlorophyll degradation, and total soluble sugar contents of cucumber grafted transplant during storage were closely related to cumulative storage temperature. However, no distinct trends showed up within changes in lipid peroxidation and

antioxidative activities in cucumber grafted transplants during storage. This result could be due to the fact that more than two abiotic stresses affected cucumber grafted transplant at the same time so that each changes in plant may be overlapped. According to these results, cucumber grafted transplant can be stored at 6-18^{o}C in continuous darkness until 3 days. To extend storage period, however, other controls of storage environmental conditions like irradiation should be added.

Keywords: chilling stress, continuous dark condition, cucumber grafted transplant, transplant storage, transplant quality

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INTRODUCTION

Cucumber (*Cucumis sativus* L.) is creeping plant of the Cucurbitaceae family. It is a warm-season, vine climbing and shallow-rooted plant and grows best at 22-29^{o}C, and originally from Southern Asia, but now grows on most continents (Encyclopaedia Britannica, 2014; RDA, 2013). Cucumber is cultivated for its fruits and one of the most popular greenhouse vegetable crops worldwide and traded on global market (Mohammadi and Omid, 2010), and global cultivation area of cucumber was 65,120,000 ton, 2012 (FAO, 2012). In Korea, cucumber cultivation area accounted for 7.8% of the total fruits vegetables growing area and cultivated on 3,629 ha and production amount was 34,209 ton, and 78% of that took place at greenhouses (Statistics Korea, 2013). Now in Korea, 89% of cucumber transplants are grafted by transplant producers (Jang et al., 2013).

Since 1990s, the increasing cultivation area of greenhouse made it possible year round production in Korea. This change in vegetable production conditions with decreasing labor force and increasing demand of transplant production have accelerated continuous increase in professional transplant producers (Park et al., 2011).

Following this trend, transplant storage technique is needed to coordinate

the supply of transplants, labor management, flexible crop scheduling, and transport over long distance (Ding et al., 2011; Kaczperski and Armitage, 1992; Kaczperski et al., 1996; Kubota and Kozai, 1995; Kubota et al., 2002). Low temperature conditions in continuous darkness have been not only widely used for maintaining quality of harvested horticultural products but also can be used for preserving transplant for a short-term period (Ding et al., 2011; Koranski et al., 1989; Kozai et al., 1996; Kubota and Kozai; 1994; Lange and Royal, 1991). Not many further researches about transplant storage technique, however, have been carried out, and the information of transplant storage is very limited.

To develop the transplant storage techniques, the deterioration process of each horticultural crop transplants under low temperature storage conditions in continuous darkness is required to be investigated. This study may be the first step to develop commercially applicable storage techniques for cucumber grafted transplants.

LITERATURE REVIEW

Plug transplant production

Separation of professional transplant production from the final crop production is a recent trend worldwide (Kubota and Kroggel, 2006). Production of high-quality transplants is crucial to determine success of final crop production both in open fields and greenhouses. The high-quality transplant production requires special techniques such as grafting or vegetative propagation, and unique facilities (Kubota et al., 1997).

Plug transplant production have been used extensively for bedding plants as well as for vegetables (Sato et al., 2004). The great advantage of plug transplant production is that the root system remains undisturbed or intact during growing and transplanting. It also allowed growers to refine production conditions to provide the optimal environment based on growth stages of transplants (Cantliffe, 2009). Other advantages of plug transplant production are reduced seed consumption, less time and labor to transplant, faster crop establishment, uniformity of plant growth, reduced disease spread, and increase in yield (Cantliffe, 2009; RDA, 2008).

Disadvantages of plug transplant production include increase in cost, requirement of highly trained workers and special facilities (Lee, 1994).

Therefore, to producers, it is critical to maximize efficiency of labors and facilities for plug transplant production. Namely, it is necessary to fill the space of facilities and to evenly distribute the workload at any time of year.

Grafted transplant

Grafting is a unique horticultural technique whereby tissues from one plant (scion) are inserted into or attached with those of another plant (rootstock) so that the two sets of vascular tissues join together and this vascular joining is called inosculation (Lee and Oda, 2003). Vegetable production with grafted transplants was started in Korea and Japan in the late 1920s (Ashita, 1927; Yamakawa, 1983), and introduced to Western countries in the early 1990s. Now grafting is being globally practiced using local scion cultivars and introduced rootstocks (Lee et al., 2010). Although accurate statistics are unavailable, rapid increases in the use of grafted transplants are expected throughout the world some decades (Davis et al., 2008; Lee and Oda, 2003; Oda, 2007).

Grafting can enhance natural resistance of scions by using that of rootstocks, including low or high temperature tolerance (Rivero et al., 2003; Venema et al., 2008; Yamakawa, 1983), quality, and yield increase (Lee and Oda, 2003), extension of harvest period (Asao et al., 1999), tolerance to soil-borne diseases such as that caused by *Fusarium*, *Verticillium*, *Phytophthora*, *Pseudomanas*,

Didymella, *Monosporascus*, and nematodes (Blestos et al., 2003; Cohen et al., 2000, 2005, 2007; Crinò et al., 2007; Edelstein et al., 1999; Ioannou, 2001; Morra and Bilotto, 2006; Trionfetti Nisini et al., 2002), and even be used for bioassay of virus infection (Davis et al., 2008; Lee and Oda, 2003). At present in Korea and Japan, most of the watermelons [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], oriental melons (*Cucumis melo* var. makuwa Makino), greenhouse cucumbers (*Cucumis sativus* L.), and several transplants of solanaceous crops are grafted (Ito, 1992; Kurata, 1992; Ryu et al., 1973).

Selection of rootstock, grafting operation, post graft handling, labor management, and uniformity of transplants with, reasonable price are the key points for business of grafted transplant production (Lee, 1994; Lee et al., 2010).

Transplant storage under low temperature and continuous dark conditions

During transplant storage, it is required to suppress stem elongation and further development and to maintain vigor of the transplants. Short- or long-term transplant storage techniques are useful in supplying plug transplants that have seasonal market demands, since holding them for a few weeks before transplanting allows flexible crop scheduling and labor management (Kubota and Kozai, 1995; Kubota et al., 2002; Sato et al., 2004).

The most common method of preserving transplant for short term period is

low temperature storage in continuous darkness (Kaczperski and Armitage, 1992), because lowering the temperatures slows metabolic processes such as photosynthesis and respiration to balance the decrease in sugar production (Kubota et al., 2002) and continuous dark condition is more commercially feasible than irradiation condition (Koranski et al., 1989). Hence low temperature storage in darkness is used as a holding method for numerous bedding plant species (Lange and Royal, 1991), as a method to extend the subculture time of some tissue-cultured plants (Mullin and Schege, 1976), or to preserve quality in many kinds of transplants (Kaczperski and Armitage, 1992; Kaczperski et al., 1996; Leskovar and Cantliffe, 1991).

Although transplant storage under continuous dark and low temperature conditions may preserve transplant vigor and inhibits overgrowth, it reduces transplant quality to some extent. Even though that defining transplant quality is very complicate, because so many factors are associated with quality evaluation and these factors influence transplant performance after transplanting (Lee et al., 2010), transplants stored under continuous darkness usually induces succulent growth, degrades chlorophyll, and reduces carbohydrate reserves (Ding et al., 2011; Rajapakse et al., 1996).

Deterioration of transplant during storage under dark and low temperature conditions

Reduction of plant quality and poor regrowth after transplanting is the problems often encountered with low temperature storage under continuous dark conditions (Koranski et al., 1989; Kubota et al., 1997). The interruption of photosynthesis under dark and low temperature conditions were regarded to be the important factors affecting the physiological changes of the transplants during storage (Ning et al., 2006).

An unfavorable dark condition during storage induced chlorophyll loss, leaf abscission, and succulent growth (Feierabend, 1977). Also when plants were exposed to low temperature conditions, chlorophyll biosynthesis was inhibited by inactivation of the involved enzymes (Hasselt and Strikwerda, 1976). Jensen et al. (1998) reported that hypocotyl elongation and succulent growth under continuous dark conditions was a complex developmental process regulated by many factors related to the roles of auxin, gibberellin, and ethylene, genetic evidences for an essential role of brassinosteroids, various photoreceptors, and the complex set of overlapping processes. Futhermore, they were ultimately regulated through the activity of multiple photoreceptors and other environmental sensory systems.

Low temperature condition may cause chilling injury during transplant storage with the symptoms of stunted growth, reduced photosynthetic capacity, necrosis, discoloration, and increased disease susceptibility (Nishida and Murata, 1996). The chilling injury can also lead to the overproduction of reactive oxygen species (ROS) exacerbating imbalance by inhibiting Calvin-Benson cycle activity (Logan et al., 2006), enhancing photosynthetic electron flux to O_2, and causing over reduction of respiratory electron transport chain (Sharma et al., 2012). Accumulation of ROS, including H_2O_2 and $O_2^{\cdot -}$ can cause lipid peroxidation and protein oxidation, which is known as a significant factor in relation to chilling injury in plants (Fryer et al., 1998; Prasad, 1997; Zhang et al., 2008).

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CHAPTER 1

Comparison of Cucumber Grafted Transplants Quality as Affected by Storage Period under Low Temperature and Continuous Dark Conditions

INTRODUTION

Low temperature conditions in darkness have been widely used for maintaining quality of harvested horticultural products, in vivo vegetative propagation material (Kubota et al., 1997), and also could be applicable to transplant storage technique. However, these storage conditions are unfavorable to transplants since the reduction of transplants quality is encountered during and after storage (Conover, 1976; Curtis and Rodney, 1952; Davis and Potter, 1985; Hansen et al., 1978). Unlike other horticultural products, transplants are growing organisms so transplant storage techniques are required to hold growth and prevent vigor loss of transplants (Kubota et al., 2002).

Under continuous dark and low temperature conditions, the main problems observed during storage were etiolated growth, chlorophyll degradation, and poor regrowth of transplants after transplanting (Ding et al., 2011; Kubota et al., 1997; Rajapakse et al., 1996). However the changes in transplants that occur

during storage under continuous dark and low temperature conditions are species specific, therefore those are need to be evaluated for each species (Justus and Kubota, 2010).

The objectives of this chapter were to investigate the changes in quality of cucumber grafted transplants during storage under continuous dark and low temperature conditions and regrowth after storage, and to determine effect of the storage temperature and storage period on post-storage transplant performance.

MATERIALS AND METHODS

Plant materials and chamber treatment conditions

Seeds of cucumber (*Cucumis sativus* L., cv. Eunsung baekdadaki; Hung-nong Seeds Co., Seoul, Korea) scion and pumpkin (*Cucurbita ficifolia*, cultivar Heukjong; Dongbu Farm Hannong Co., Seoul, Korea) rootstock were sown and grafted at Poseung Horti-Agriculture Association (36°58'N, 126°52'E), Pyeongtaek, Korea. Both scions and rootstocks were sown on the same day (Feb. 25, 2014) and grafted 15 days after sowing. The grafted transplants were grown for 25 days and packaged in cardboard boxes which were conventionally used for transplant transportation and transported (took 80 min) to Seoul National University, Seoul, Korea. The packed boxes with transplants were stored under five different conditions (Fig. 1-1). The temperatures and relative humidity in the chambers were maintained at 6, 9, 12, 15, and 18^{o}C, and 85-95%, in darkness, respectively. The transplants have been stored in each chamber for 3, 6, 9, 12, and 15 days and measured plant height, SPAD value. Then eight transplants for each treatment were taken from the chambers and transplanted into plastic pots filled with a commercial substrate containing fertilizer (Sikmulsekye; Nongwoo Bio Co., Suwon, Korea) in the greenhouse at Experimental Farm (37°16’N, 126°59’E) of Seoul National University, Suwon, Korea. For 15 days treatment,

additional eight transplants were left in the greenhouse and grown without storage as the control. Overhead irrigation was daily applied with tap water for 15 days of cultivation. After 15 days of storage, several growth parameters including percent survival, plant height, SPAD value, number of leaves, leaf area, number of female flowers, and fresh and dry weight of shoots and roots of cucumber grafted transplants were determined.

Statistical analysis

Data were analyzed using SAS 9.3 version (SAS Inst. Inc., Cary, NC, USA) for ANOVA and Duncan's multiple range test (DMRT) at $P \leq 0.05$.

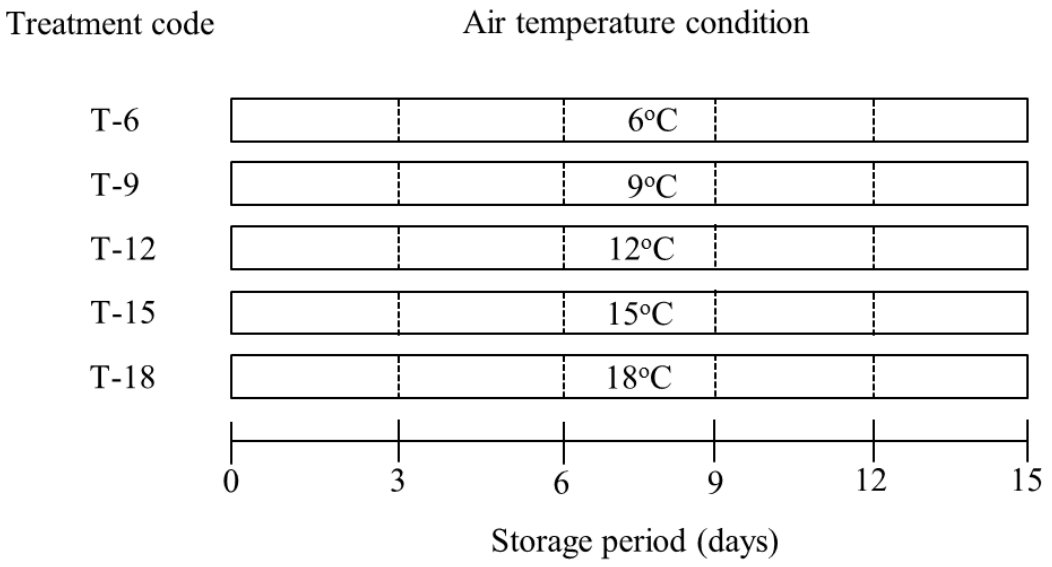

Fig. 1-1. Treatment codes for various air temperature conditions in continuous darkness.

RESULTS AND DISSCUSSION

Changes in plant height and chlorophyll contents during storage

After 15 days of storage, the plant height of cucumber grafted transplants in T-6, T-9, T-12, T-15, and T-18 was 6, 10, 17, 37, and 50% longer, respectively, than that of cucumber grafted transplants before storage (Fig. 1-2). This increase of plant height occurred markedly for the first 3 days of storage. The plant height of cucumber grafted transplants in T-6, T-9, T-12, T-15, and T-18 stored for 3 days was 3, 6, 13, 20, and 41% longer than that of cucumber grafted transplants before storage. After 3 days of storage, the rate of stem elongation was declined in all treatments until the end of the storage.

No significant differences in SPAD values of cucumber grafted transplants were observed among all treatments for the first 3 days after storage (Fig. 1-3), but during the storage the SPAD value decreased more rapidly compared to that after 3 days of storage. After 3 days of storage, SPAD values decreased continuously in T-18 treatment until the end of storage. After 15 days of storage, the SPAD values were greater in treatments set at relatively high air temperatures. In T-6, T-9, T-12, T-15, and T-18 treatments stored for 15 days, the SPAD values decreased 14, 18, 23, 29, and 52%, respectively, compared to that of cucumber grafted transplants before storage.

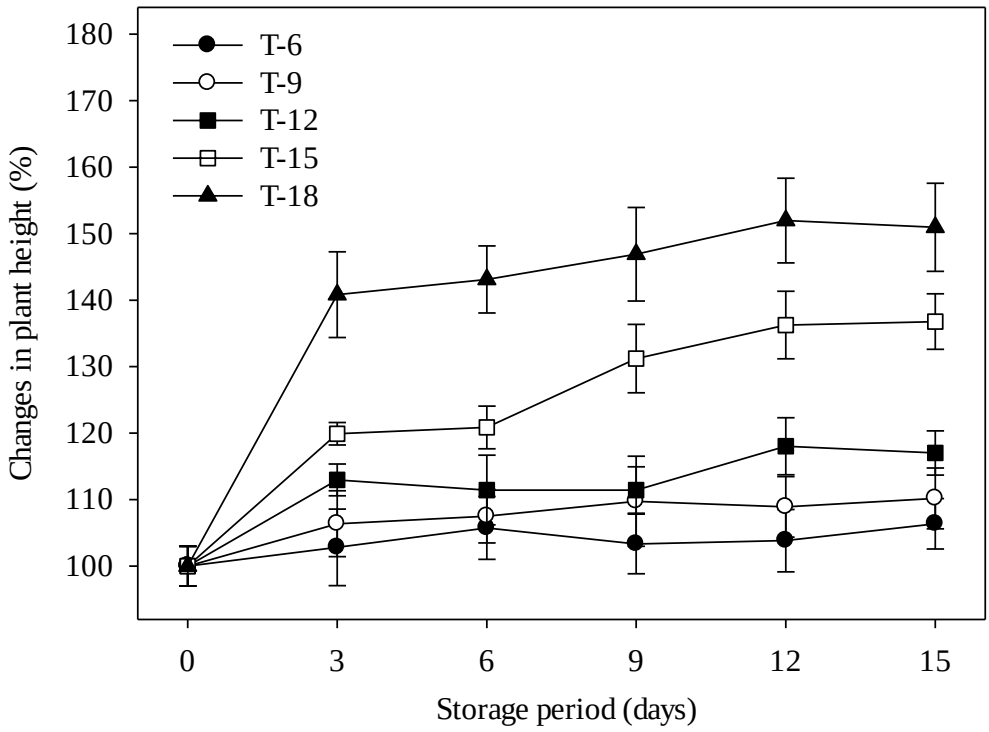

Fig. 1-2. Changes in plant height of cucumber grafted transplants stored under various air temperature conditions for 15 days.

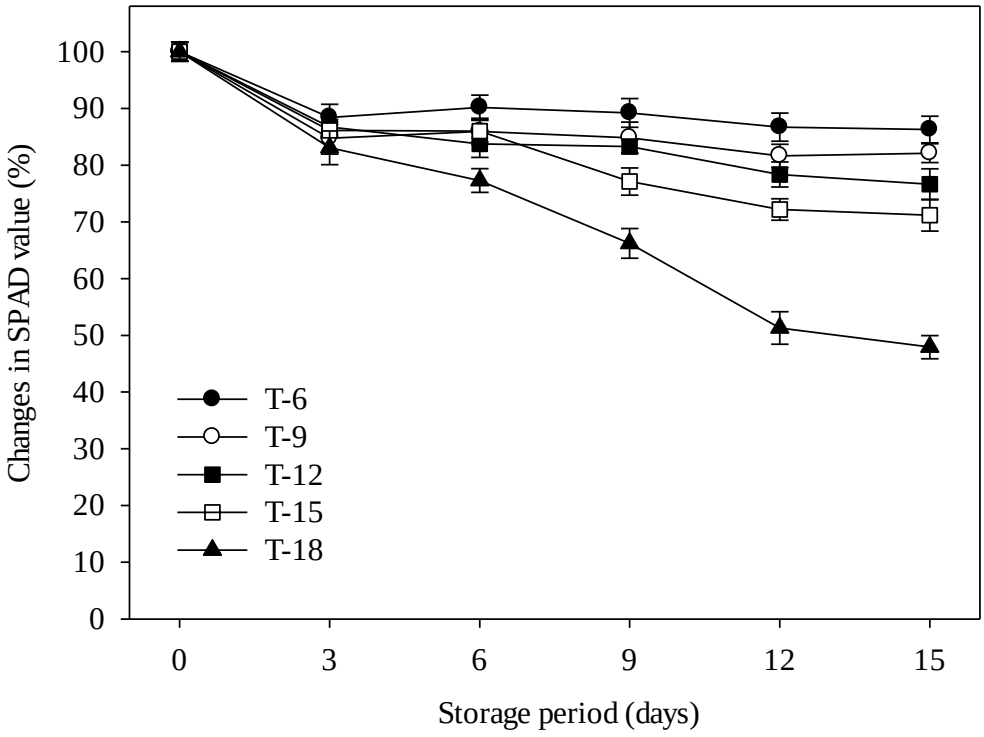

Fig. 1-3. Changes in SPAD value of cucumber grafted transplants stored under various air temperature conditions for 15 days.

Stem elongation during storage was a consequence of the environmental conditions in which the growth of transplants was not completely suppressed (Heins et al., 1995). During storage in continuous darkness, succulent growth of transplants could be inevitable. Even though both light and hormones like giberellins control hypocotyl and mesocotyl elongation in cucumber (Boylan and Quail, 1989; Goto et al., 1993; Koornneef et al., 1980, 1985; Liscum and Hangarter, 1991; Lopez-Juez et al., 1995; McNellis et al., 1994), and phytochrome and cryptochrome photoreceptors also controlled the rate of hypocotyl elongation (Parks et al., 2001), the storage air temperature in this condition was the main factor affecting stem elongation of cucumber grafted transplant. The low temperature condition could reduce the metabolic processes such as respiration and hormone and enzyme activities, and thus succulent growth of transplants might be reduced during storage. Air temperature condition of 6^{o}C in continuous darkness induced the least changes in plant height of cucumber grafted transplants during storage (Fig. 1-2).

Lichtenthaler et al. (1981) reported that chlorophyll and mesophyll degradation were the natural physiological responses for plants in response to low light environments, and storage temperature and rate of chlorophyll breakdown in this study might be correlated. Increasing air temperature and duration of storage period accelerated decreasing chlorophyll concentration of

transplants during low temperature storage in continuous darkness as reported by Justus and Kubota (2010).

Growth of cucumber grafted transplants after transplanting

Transplants in all the treatments stored for 3 days survived after transplanting. No significant differences were observed in regrowth after 3 days of storage among all treatments (Table 1-1). No vigor loss of transplants was observed until 3 days of storage at 6, 9, 12, 15, and 18^{o}C and continuous dark condition. However, the early growth of transplants in T-6 and T-9 treatment stored for 6 days was more greatly suppressed after storage compared to that in the other treatments. Percent survival was 100% in T-6 and T-15 treatments (Table 1-2). About the growth of transplants stored for 9 days, none of the cucumber grafted transplants in T-6 treatment survived and those in T-9 treatments showed poor growth with respect to all investigated parameters than those in the other treatments except T-6 (Table 1-3). Percent survival was 100 and 88% in T-15 treatment and other treatments, respectively.

In cucumber grafted transplants transplanted after 12 days of storage, T-6 treatment did not survive at all and T-9 treatment showed the lowest plant height and percent survival. The percent survival was decreased as 38 and 75% in T-9 and the other treatments except T-6, respectively (Table 1-4).

After 15 days of storage and transplanting, only transplants in T-12 and T-15 treatments survived, but showed low percent survival as 38 and 25% and there was not statistical significantly different between the two treatments (Table 1-5).

Long term exposure to low temperature can cause chilling injury to cucumber grafted transplant during storage. The damages of chilling injury revealed as stunted growth reduced photosynthetic capacity, necrosis, discoloration, and increased disease susceptibility on plants (Nishida and Murata, 1996). With chlorophyll degradation in leaf of transplants during storage, chlorophyll remaining in the leaves of the plantlets stored in darkness lost its ability to operate the processes of photosynthesis, the reaction-center chlorophyll of photosystem II was destroyed (Lichtenthaler, 1988). The dry weight per transplants stored in darkness decreased due to the continuous respiration that occurred in darkness. Likewise transplants stored in darkness continued to respire during storage, providing a negative carbon balance and reducing carbohydrate levels and dry weights. The decrease in respiration rates in darkness was thought to be partly due to the decrease in levels of carbohydrates that can be used as respiratory substrates in leaves and stems (Kubota et al., 1997). All of those changes in transplant during storage could be a potential threat to transplants. In addition, delay of the regrowth upon transplanting might

be avoided by having acclimation process after storage by having to adapt relatively large differences of the environments between storage and growth conditions as reported by Kubota et al. (2002).

Table 1-1. Plant height, SPAD value, numbers of leaves and female flowers, leaf area, and fresh and dry weights of non-stored and 3 days-stored cucumber grafted transplants 15 days after transplanting.

Treatment	Percent survival	Plant height (cm)	SPAD value	No. of leaves per plant	Leaf area (cm^3/plant)	No. of female flowers per plant	Fresh weight (g/plant)		Dry weight (g/plant)	
							Shoot	Root	Shoot	Root
Non-stored	100	36.8	45.3	7.4	624.5	5.5	28.3	5.2	3.1	0.4
T-6	100	38.6 a[z]	43.0 a	8.0 a	599.8 a	6.8 ab	27.5 ab	4.0 b	3.0 a	0.3 a
T-9	100	36.8 ab	43.1 a	7.9 a	611.7 a	7.3 a	26.9 b	4.9 ab	3.0 a	0.3 a
T-12	100	39.3 ab	41.8 ab	7.4 ab	599.6 a	6.3 abc	27.7 ab	5.5 a	3.0 a	0.4 a
T-15	100	41.9 a	42.3 ab	7.5 ab	644.5 a	7.4 a	32.1 a	4.8 ab	3.3 a	0.3 a
T-18	100	34.5 b	39.5 b	7.0 b	458.9 b	5.0 c	19.8 c	4.7 ab	2.2 b	0.3 a

[z]Means in columns followed by different letters are significantly different by DMRT at $P \leq 0.05$.

Table 1-2. Plant height, SPAD value, numbers of leaves and female flowers, leaf area, and fresh and dry weights of non-stored and 6 days-stored cucumber grafted transplants 15 days after transplanting.

Treatment	Percent survival	Plant height (cm)	SPAD value	No. of leaves per plant	Leaf area (cm^3/plant)	No. of female flowers per plant	Fresh weight (g/plant)		Dry weight (g/plant)	
							Shoot	Root	Shoot	Root
Non-stored	100	36.8	45.3	7.4	624.5	5.5	28.3	5.2	3.1	0.4
T-6	100	13.8 b[z]	51.3 a	4.0 b	237.3 c	1.4 c	13.4 c	2.3 b	2.0 a	0.2 b
T-9	88	13.4 b	45.5 b	6.0 a	333.7 bc	2.7 bc	15.6 c	2.8 b	2.1 a	0.2 b
T-12	88	26.1 a	44.4 b	6.6 a	462.2 ab	5.0 ab	20.5 ab	5.0 a	2.6 a	0.3 a
T-15	100	33.4 a	43.2 b	7.8 a	535.2 a	7.0 a	23.6 a	4.9 a	2.6 a	0.3 a
T-18	88	33.5 a	42.2 b	7.6 a	493.8 a	6.4 a	20.0 ab	4.9 a	2.4 a	0.3 a

[z]Means in columns followed by different letters are significantly different by DMRT at $P \leq 0.05$.

Table 1-3. Plant height, SPAD value, numbers of leaves and female flowers, leaf area, and fresh and dry weights of non-stored and 9 days-stored cucumber grafted transplants 15 days after transplanting.

Treatment	Percent survival	Plant height (cm)	SPAD value	No. of leaves per plant	Leaf area (cm^3/plant)	No. of female flowers per plant	Fresh weight (g/plant)		Dry weight (g/plant)	
							Shoot	Root	Shoot	Root
Non-stored	100	36.8	45.3	7.4	624.5	5.5	28.3	5.2	3.1	0.4
T-6	n/a[z]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
T-9	88	8.3 b[y]	51.0 a	3.5 b	120.1 b	0.8 b	7.3 b	1.5 b	1.0 b	0.1 b
T-12	88	30.1 a	41.5 b	7.3 a	455.7 a	5.4 a	19.6 a	3.9 a	1.9 a	0.3 a
T-15	100	31.2 a	46.4 ab	7.3 a	415.0 a	6.8 a	18.2 a	3.1 a	1.9 a	0.2 a
T-18	88	33.1 a	46.1 ab	7.4 a	450.6 a	5.6 a	19.6 a	2.8 ab	1.8 a	0.2 a

[z]Not available.

[y]Means in columns followed by different letters are significantly different by DMRT at $P \leq 0.05$.

Table 1-4. Plant height, SPAD value, numbers of leaves and female flowers, leaf area, and fresh and dry weights of non-stored and 12 days-stored cucumber grafted transplants 15 days after transplanting.

Treatment	Percent survival	Plant height (cm)	SPAD value	No. of leaves per plant	Leaf area (cm^3/plant)	No. of female flowers per plant	Fresh weight (g/plant)		Dry weight (g/plant)	
							Shoot	Root	Shoot	Root
Non-stored	100	36.8	45.3	7.4	624.5	5.5	28.3	5.2	3.1	0.4
T-6	n/a[z]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
T-9	38	5.3 b[y]	50.0 a	5.0 a	281.7 a	3.0 a	13.7 a	3.6 a	1.7 a	0.2 a
T-12	75	22.3 a	40.6 b	5.7 a	281.6 a	5.0 a	12.5 a	2.3 a	1.1 a	0.1 a
T-15	75	27.8 a	40.1 b	6.7 a	353.8 a	6.3 a	14.3 a	2.4 a	1.2 a	0.1 a
T-18	75	29.7 a	42.0 b	6.7 a	301.2 a	3.7 a	12.5 a	2.8 a	1.1 a	0.1 a

[z]Not available.

[y]Means in columns followed by different letters are significantly different by DMRT at $P \leq 0.05$.

Table 1-5. Plant height, SPAD value, numbers of leaves and female flowers, leaf area, and fresh and dry weights of non-stored and 15 days-stored cucumber grafted transplants 15 days after transplanting.

Treatment	Percent survival	Plant height (cm)	SPAD value	No. of leaves per plant	Leaf area (cm^3/plant)	No. of female flowers per plant	Fresh weight (g/plant)		Dry weight (g/plant)	
							Shoot	Root	Shoot	Root
Non-stored	100	36.8	45.3	7.4	624.5	5.5	28.3	5.2	3.1	0.4
T-6	n/a[z]	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
T-9	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
T-12	38	25.0 a[y]	41.4 a	6.7 a	354.0 a	4.0 a	12.9 a	1.9 a	1.3 a	0.1 a
T-15	25	33.8 a	40.4 a	8.0 a	450.5 a	7.0 a	16.6 a	1.3 a	1.5 a	0.1 a
T-18	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

[z]Not available.

[y]Means in columns followed by different letters are significantly different by DMRT at $P \leq 0.05$.

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CHAPTER 2

Physiological Changes of Cucumber Grafted Transplants Stored under Low Temperature and Continuous Dark Conditions

INTRODUCTION

In the first chapter, I found that over 3 days of storage under continuous darkness and low temperature conditions induced severe deterioration of cucumber grafted transplants. The abiotic stress caused by continuous darkness and low temperature conditions induced quality deterioration and physiological changes of cucumber grafted transplant during storage.

When plants are exposed to abiotic stress, it leads to enhanced generation of reactive oxygen species (ROS) due to disruption of cellular homeostasis, and all the ROS are extremely harmful to organisms at high concentrations (Hu et al., 2008; Mishra et al., 2011; Shah et al., 2001; Sharma and Dubey, 2005). Chilling stress under low temperature conditions leads to changes in the activities of antioxidative defense system in plants, which increases generation of $O_2^{\cdot -}$, H_2O_2 and other ROS (Kang and Slatveit, 2001, 2002; Lu et al., 2004). ROS are highly reactive and can damage membrane lipids, proteins, and nucleic acids

(Scandalios, 1993). Lipid peroxidation on the cell and membrane caused by accumulation of ROS will produce more malondialdehyde (MDA) in plant tissues (Hung et al., 2005; Shi et al., 2007). The oxidative stress induced by chilling stress during storage may play a pivotal role for deterioration of cucumber grafted transplants. The retention of carbohydrate reserves in transplants is an important factor for preserve vigor of transplants (Wilson et al., 1998), but during transplant storage loss of carbohydrate reserves in transplants are induced by respiration, because of the interruption of photosynthesis under continuous dark condition (Davis and Potter, 1985; Hansen et al., 1978).

According to the previous results of the first chapter, stem elongation and chlorophyll degradation of cucumber grafted transplants was suppressed for 3 days of storage at around 6^{o}C. A hypothesis, that gradually increasing air temperatures during storage could reduce chilling stress and damages of cucumber grafted transplants and the storage period could be extended, was set up and examined in chapter 2.

The objective of this chapter was to investigate the changes in quality of cucumber grafted transplants during storage under continuous dark and constant low temperature and gradually increasing conditions. In addition, changes in lipid peroxidation and antioxidant capacity of the cucumber grafted transplants affected by these storage conditions during storage were also investigated.

MATERIALS AND METHODS

Plant materials and storage conditions

Seeds of cucumber (*Cucumis sativus* L., cv. Joen baekdadaki; Hung-nong Seeds Co., Seoul, Korea) scion and pumpkin (*Cucurbita ficifolia*, cv. Shintozwa; Nongwoo Bio Co., Suwon, Korea) rootstock were sown and grafted by Poseung Horti-Agriculture Association Co. (36°58'N, 126°52'E), Pyeongtaek, Korea. Both scions and rootstocks were sown on the same day (Sep. 06, 2014) and grafted 7 days after sowing. The grafted transplants were grown for 20 days and packaged in cardboard boxes which were conventionally used for transplant transportation and transported (took 80 min) to Seoul National University, Seoul, Korea. The four different chambers set at 6, 10, 14, and 18^{o}C and the packed boxes with transplants were stored in the chambers. The air temperature and relative humidity were monitored using T-type thermocouples and wet and dry bulb thermometers connected to a data logger (CR 10X, Campbell Scientific, Logan, UT, USA). Fig. 2-1 showed treatment codes for various storage conditions. During 15 days treatment period, the transplants in each treatment were measured with respect to plant height and SPAD value, and sampled every 3 days for the assay. Each sample was stored at −80^{o}C until assay. Before assay, frozen samples were lyophilized and ground using a mill (Thomas Wiley Mini

Mill 3383-L10, Thomas Scientific, Swedesboro, NJ, USA) with a 40-mesh screen.

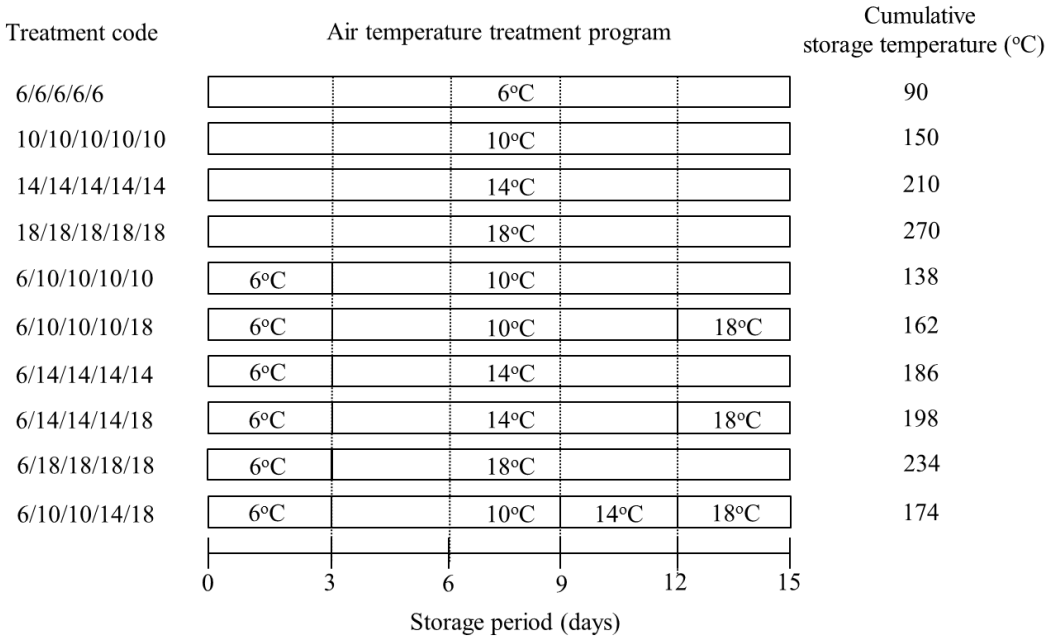

Fig. 2-1. Treatment codes for constant and increasing air temperature conditions in continuous darkness.

Determination of lipid peroxidation

The level of lipid peroxidation was measured as the amount of MDA which was determined by the thiobarbituric acid (TBA) reaction (González et al., 1995; Heath and Packer 1968; Lee et al., 1998). Fifty milligrams of lyophilized sample powder in 2.5 ml of triple distilled water (TDW) were added with 2.5 ml of 0.5% TBA in 20% trichloroacetic acid. The mixture was heated for 30 min a water bath at 95^{o}C and then the reaction was stopped by putting the reaction tube an ice bucket for 15 min. The reaction tubes were centrifuged at 2,500 g for 5 min and then the 2 ml of supernatants were collected. Following centrifugation at 20,000 g for 15 min, the resulting supernatants were used for spectrophotometric determination of MDA. The absorbance of supernatant was determined at 532 nm for each sample and corrected for nonspecific turbidity at 600 nm. MDA concentration was calculated using a molar extinction coefficient of 155 mM^{-1} cm^{-1} by using the formula:

$$\text{MDA content } (\mu\text{mol}) = \{(A_{532} - A_{600})/155\} \times 10^{3}$$

where A_{532} is absorbance at 532 nm, and A_{600} is absorbance at 600 nm.

Determination of antioxidant activity using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity assay was performed as described by Fukumoto and Mazza (2000) with some modifications. Fifty milligrams of lyophilized sample powder was suspended in 5 ml methanol and ultrasonicated for 2 h. The mixture was then filtered through an 8 μm cellulose of the filter paper (Whatman Intl. Ltd., Kent, UK) for 5 min. DPPH solution was prepared at a concentration of 150 μM in 80% methanol and 200 μl of this solution was added into each well of a 96-well plate. To 25 μl sample extraction, standard solutions were added into each well with DPPH solution and mixed. The plates were covered and allowed to react in the dark at room temperature for 12 h. The absorbance at 517 nm was determined with a microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA, USA) and compared to standard curve. Each assay was carried out in triplicate and the 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) was used to generate a standard curve at 10-250 μM.

Determination of total soluble sugar (TSS)

Determination of TSS was performed as described by Smith and Zeeman (2006) with some modifications (Lee et al., 2012; Lee et al., 2013). Fifty milligrams of lyophilized sample powder were extracted in 1 ml of 80% (v/v) ethanol and incubated at 80^{o}C for 15 min, and then centrifuged at 15,000 g for 10

min. The supernatant was transferred to a conical tube, and the pellet was extracted twice more as above. The supernatant was evaporated at 70°C using a N_2 evaporator, and after conical tube was filled with 1.5 ml TDW and ultrasonicated to dissolve thoroughly. The sugars melted solution was passed through Sep-Pak C18 cartridge (Waters Division, Millipore, Milford, MA, USA) and 0.45 μl syringe filter (Whatman Intl. Ltd., Kent, UK) using 3 ml syringe. Sugars in samples were separated in a high-performance liquid chromatography (Dionex Ultimate 3000 HPLC system, Dionex, Sunnyvale, CA, USA; Shodex RI-101 refractive index detector, Showa Denko, Tokyo, Japan; Sugar-Pak I column 300 × 6.5 mm, Waters Division, Millipore, Milford, MA, USA) under the following conditions: sample volume, 10 μl; mobile phase, TDW; flow rate, 0.5 ml min^{-1}; and column temperature, 80°C.

Statistical analysis

Data were analyzed using SAS 9.3 version (SAS Inst. Inc., Cary, NC, USA) for ANOVA and Duncan's multiple range test (DMRT) at $P \leq 0.05$.

RESULTS AND DISSCUSSION

Changes in plant height and chlorophyll contents during storage

In 18/18/18/18/18 treatment, the plant height of cucumber grafted transplants markedly increased, but there were no significant changes in that of the other treatments for the first 3 days after storage. At constant air temperature conditions of 6, 10, 14, and 18^{o}C, the plant height of cucumber grafted transplants after 15 days of storage increased 2, 3, 7, and 24% compared to that of cucumber grafted transplants before storage, respectively (Fig. 2-2).

The six combination treatments consisted of increasing air temperature conditions, after 3 days of storage at 6^{o}C, cucumber grafted transplants in the five treatments except 6/18/18/18/18 showed 2% of stem elongation compared to the plant height of cucumber grafted transplants before storage until the end of 15 days of storage. After 3 days of storage at 6^{o}C, however, cucumber grafted transplants in 6/18/18/18/18 treatments showed continuously increasing trend of stem elongation and finally the plant height increased by 14% compared to that of cucumber grafted transplants before storage.

Fig. 2-3 showed these trends in stem elongation of cucumber grafted transplants were exponentially related to cumulative storage temperature by cumulative storage temperature inducing stem elongation during storage. There was no

significant difference in SPAD values of cucumber grafted transplants among 3 treatments consisted of constant air temperature conditions during first 3 days of storage except treatment 18/18/18/18/18 (Fig. 2-4). At the constant air temperature, the SPAD values of cucumber grafted transplants decreased continuously until the end of storage. At constant air temperatures of 6, 10, 14, and 18^{o}C, SPAD value of cucumber grafted transplants decreased reaching to 14, 26, 36, and 66% compared to that of cucumber grafted transplants before storage. The six treatments consisted of increasing air temperature conditions, the SPAD values of cucumber grafted transplants almost linearly decreased regardless of changes in air temperature during storage. Fig. 2-5 showed that these changes were linearly related to cumulative storage temperature.

Hypocotyl elongation was thought to be a complex developmental process regulated by many factors (Jensen et al., 1998), and it was hard to find out what physiological factors caused stem elongation in this study. However, results suggest that stem elongation of cucumber grafted transplant during storage under low temperature and continuous dark conditions may be significantly influenced by cumulative storage temperature not by the daily changes in storage temperature. Within the natural environment, continuous darkness and both high and low temperatures have been implicated in the destruction of chlorophyll (Schoch et al., 1984). Chlorophyll content in plants often changes in parallel with

chlorophyllase activity and the chlorophyllase has been assumed to participate in the biosynthesis and degradation of chlorophyll (Chiba et al., 1967; Holden, 1961; Shimizu and Tamaki, 1962). Chlorophyllase activity differed depending on temperature changes (Ogura, 1972), so that the changes in storage temperature may have affected the chlorophyll degradation during storage.

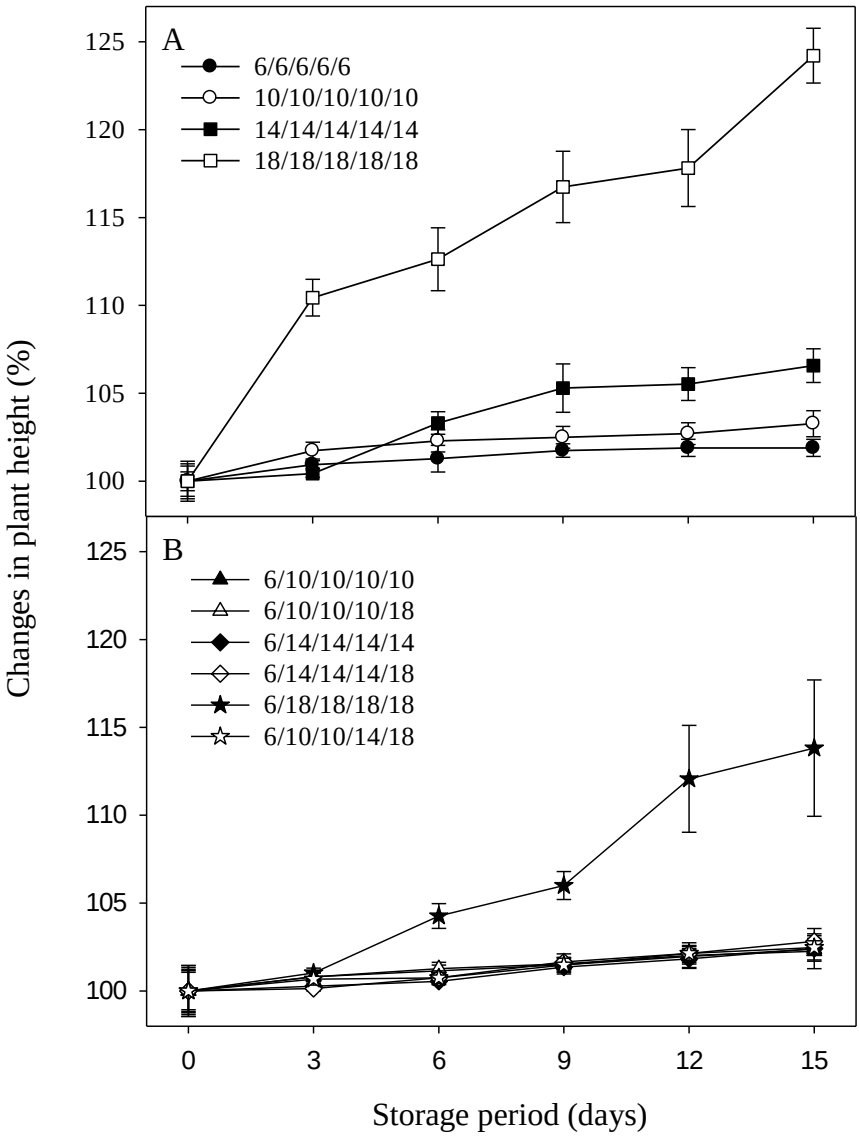

Fig. 2-2. Changes in plant height of cucumber grafted transplants stored under constant (A) and increasing (B) air temperature conditions in continuous darkness for 15 days. Plant height before storage was considered 100%.

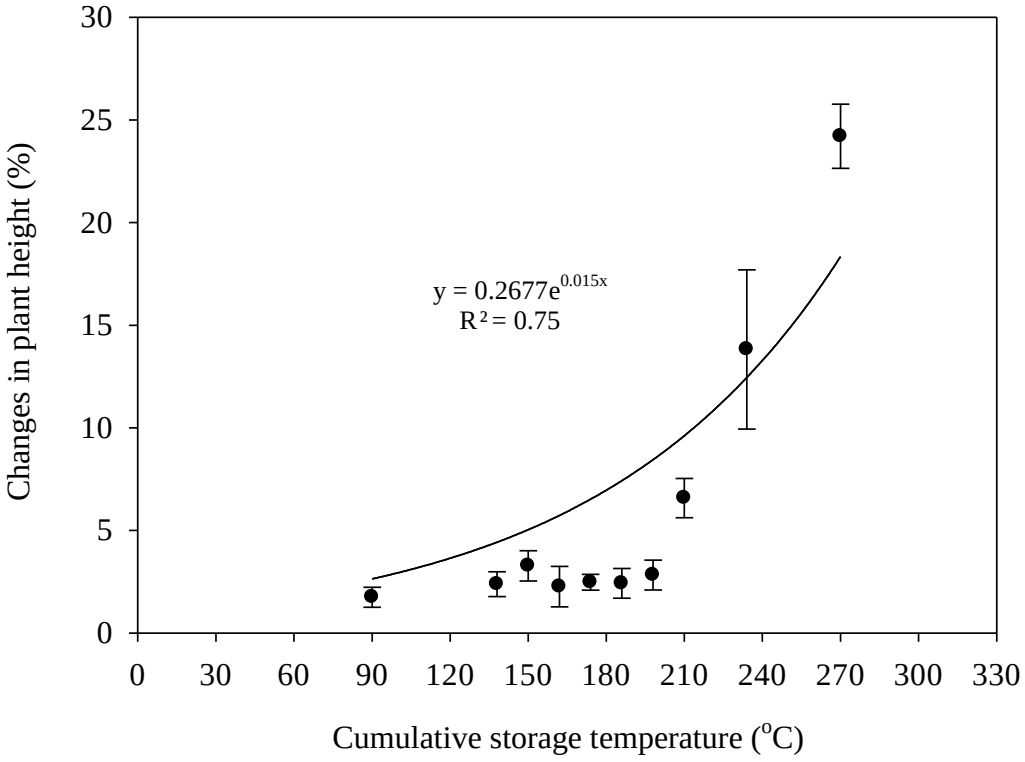

Fig. 2-3. Relationship between stem elongations of cucumber grafted transplants and cumulative storage temperatures for 15 day. Plant height before storage was considered 0%.

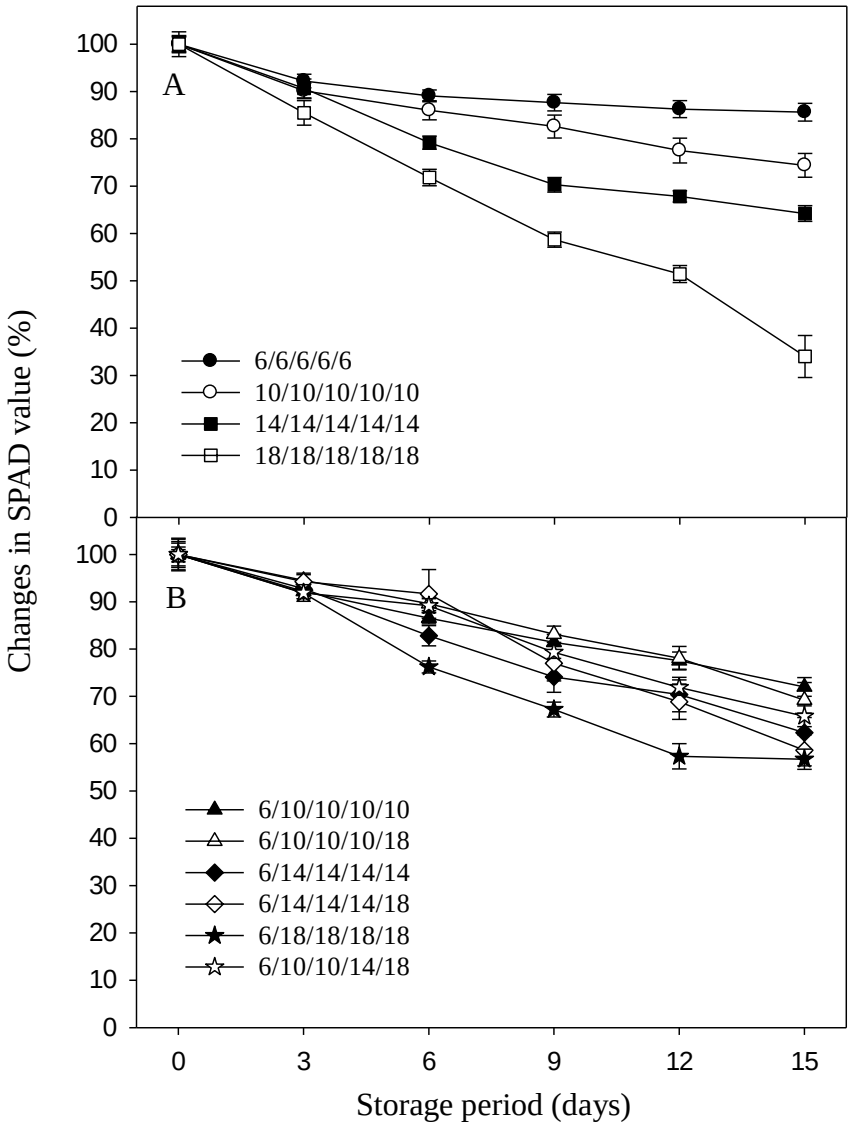

Fig. 2-4. Changes in SPAD value of cucumber grafted transplants stored under constant (A) and increasing (B) air temperature conditions in continuous darkness for 15 days. SPAD value before storage was considered 100%.

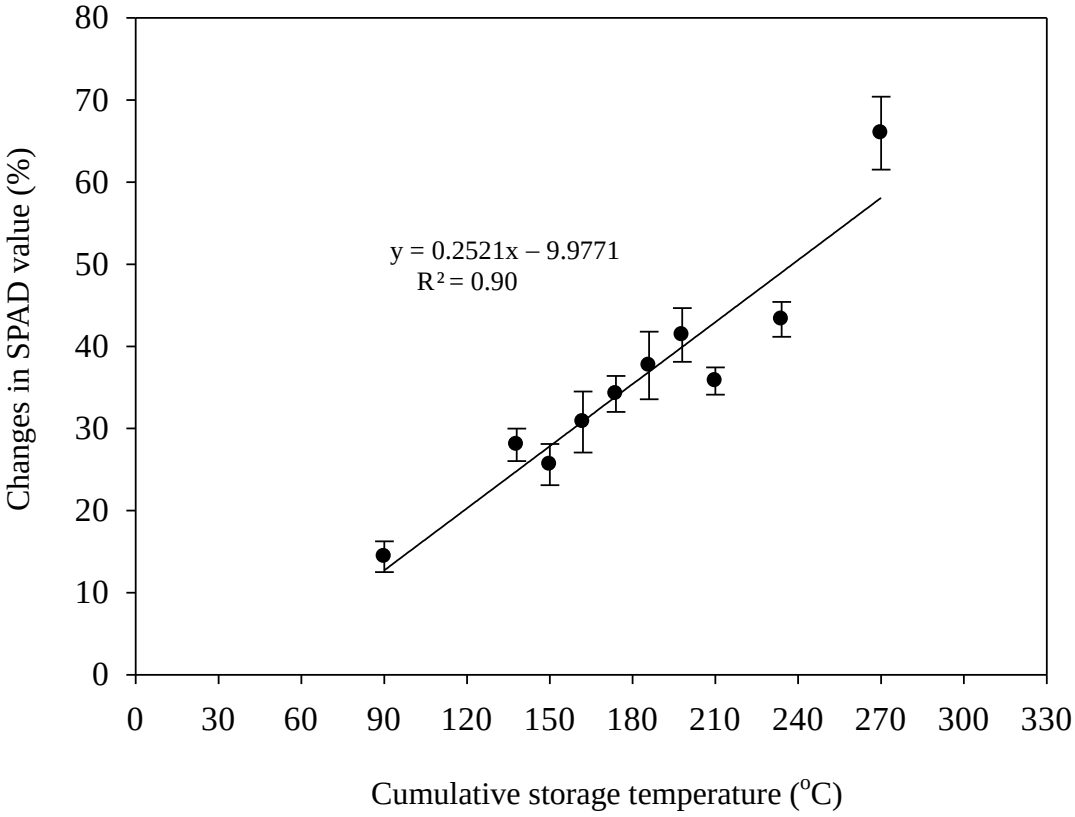

Fig. 2-5. Relationship between decreases in SPAD value of cucumber grafted transplants and cumulative storage temperatures for 15 days. SPAD value showed reduced rate compared to that of before storage.

Changes in MDA content and DPPH radical scavenging activity during storage

MDA is the ultimate product of membrane lipid peroxidation, and its content is closely related to the damage extent of ROS (Asada et al., 1998; Salin, 1988; Wang et al., 2004). The MDA contents of cucumber grafted transplants during storage were increased at the end of storage in comparison with those of cucumber grafted transplants before storage, but no certain trend was found in the four treatments consisted of constant air temperature conditions (Fig. 2-6). At 3 days of storage when the storage temperatures were changed, MDA contents increased in the six treatments of increasing air temperatures. This result suggests that increasing storage temperature after 3 days storage at 6^{o}C did not induce recovery of cucumber grafted transplants.

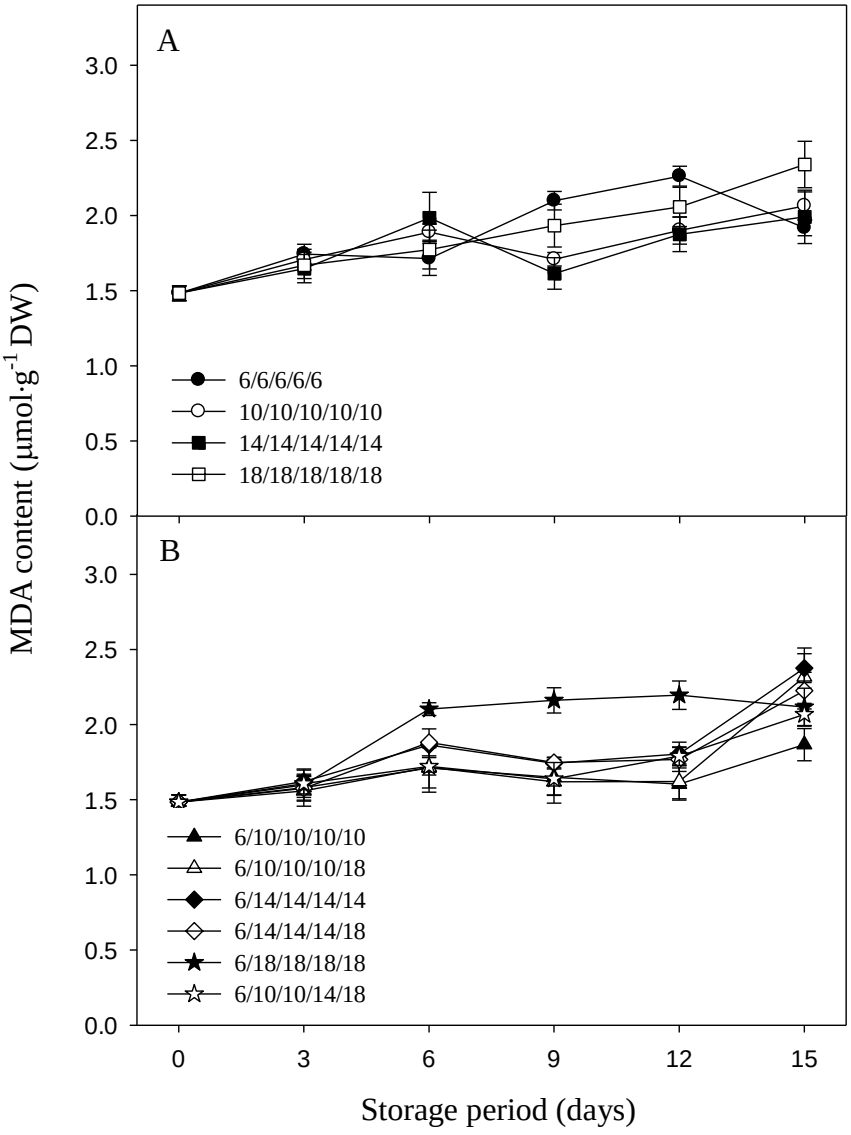

Fig. 2-6. Changes in MDA content of cucumber grafted transplants stored under constant (A) and increasing (B) air temperature conditions in continuous darkness for 15 days.

The DPPH radical scavenging activity assay offers a possibility to estimate the level of radical scavenging antioxidants by investigating the bleaching of DPPH (Malterud et al., 1993). In this study, however, the changes in DPPH scavenging activity of all treatment showed no certain trend during storage (Fig. 2-7).

The DPPH assay is limited to the detection of certain radical scavenging antioxidants like ascorbate. Thus, enzyme antioxidants like superoxide dismutase, catalase, and glutathione peroxidase or metal chelating antioxidants like polyamines, curcumin, desferal or similars will escape detection by the DPPH assay and some radical scavenging antioxidants like glutathione are not detected by the assay (Løvaas and Olsen, 1998). And if two or more abiotic stresses affected cucumber grafted transplant at the same time, that may make it hard to find certain trend in the results of MDA and DPPH assay.

Decrease in TSS content after storage

TSS in cucumber grafted transplants consumed during dark storage (Kubota et al., 1997). The decrease in TSS contents of stored cucumber grafted transplants compared to that of cucumber grafted transplants before storage had correlation with cumulative storage temperatures (Fig. 2-8). Decrease in TSS increased with increasing cumulative storage temperatures.

The carbohydrate reserves in the transplants are important for growth during storage (Wilson et al., 1998). The maintenance of a higher level of soluble sugar may enhance tolerance against low temperature (Ma et al., 2009) and affect the survival ratio and photosynthetic recovery of transplants after transplanting (Wilson et al., 1998). To maintain the vigor of transplant during storage, it may be important to control the consumption of soluble sugars in transplants followed by changes of storage temperature.

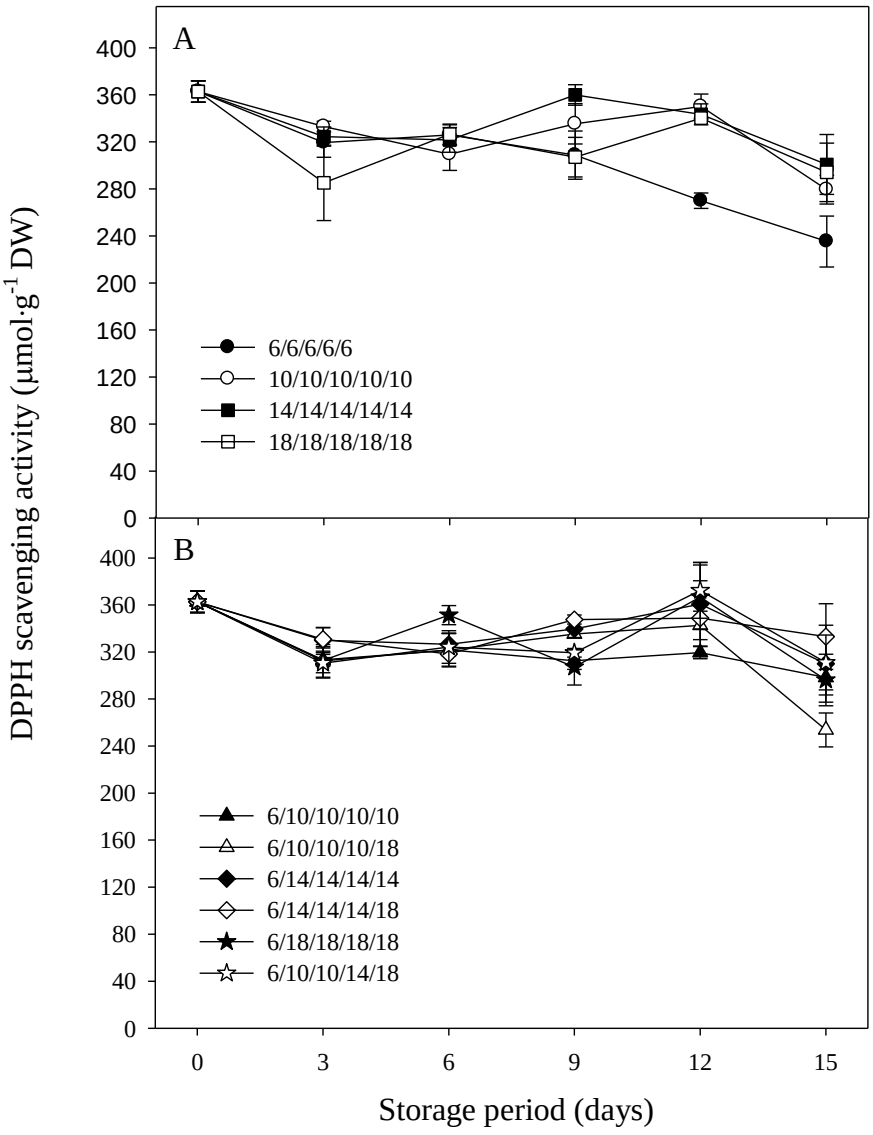

Fig. 2-7. Changes in DPPH-radical scavenging activity of cucumber transplants stored under constant (A) and increasing (B) air temperature conditions in continuous darkness for 15 days.

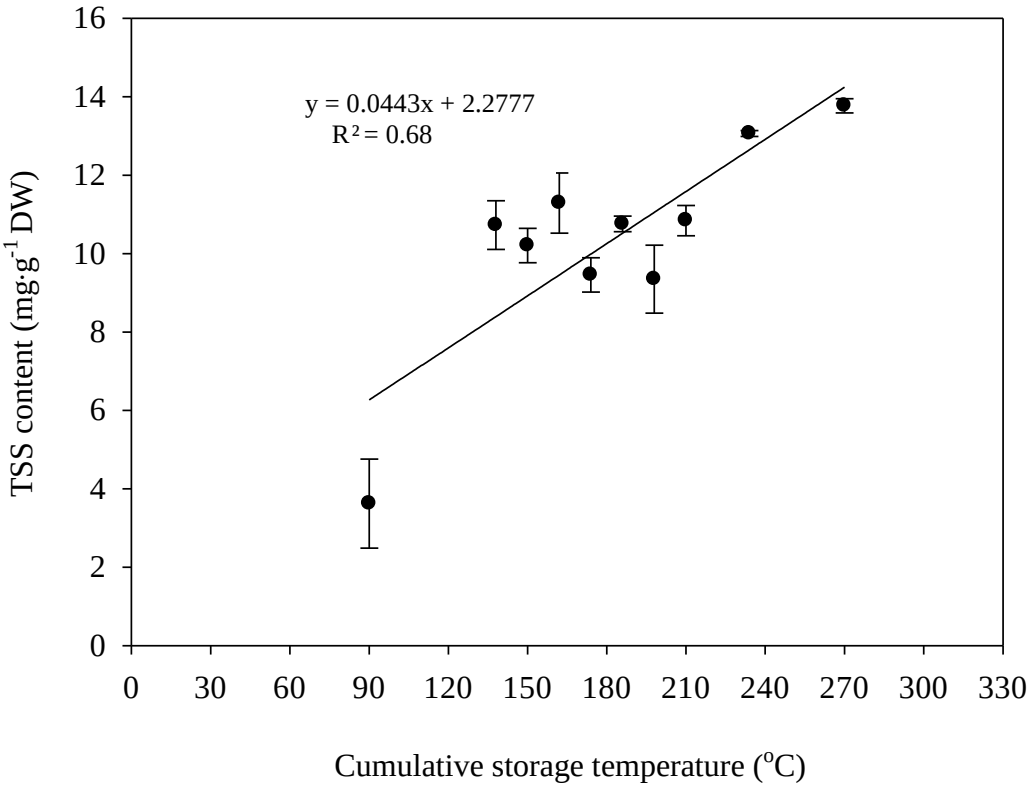

Fig. 2-8. Relationship between decreases in TSS content of cucumber grafted transplants and cumulative storage temperatures after 15 days. TSS content showed the amount reduced compared to that of before storage.

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CONCLUSION

Cucumber grafted transplant stored at 6, 9, 12, 15, 18^{o}C under continuous dark condition can be stored for 3 days without vigor loss during storage. For the first 3 days of storage, decrease in SPAD values during storage was not significantly affected by storage temperature, but plant height increased rapidly as storage temperature increased. As storage period in darkness lengthened and as storage temperature increased; stem elongation, chlorophyll degradation and loss of vigor for regrowth after transplanting increased. Such changes resulted in reduction of percent survival after transplanting.

If cucumber transplant stored at 6^{o}C for 3 days, stem elongation could be suppressed even if storage temperature increase to some extent after 3 days of storage. Stem elongation and chlorophyll degradation during cucumber grafted transplant storage at low temperature under continuous dark condition might be affected by activity of related enzymes that varies depending on air temperature. Decrease in TSS content in cucumber grafted transplant could impair tolerance against low temperature and affected percent survival after transplanting. Stem elongation, chlorophyll degradation and consumption of TSS content in cucumber grafted transplants were also highly related to cumulative storage temperature, more than momentary changes in air temperature during storage.

The physiological changes derived from abiotic stress in cucumber grafted transplant during storage could not be clearly evaluated in this study, but it could be hypothesized that several stresses affected cucumber grafted transplants during storage at the same time.

Through the control of storage air temperature, deterioration of quality of cucumber grafted transplant could be minimized during storage but they could not be successfully stored over 3 days under continuous dark condition. Hence to extend storage period or develop commercially applicable transplant storage techniques, other environment control technologies e.g., supplementing irradiation, humidity control and others might be considered.

ABSTRACT IN KOREAN

본 연구에서는 공정묘의 안정생산을 위한 저장기술 개발을 위하여 가장 보편적으로 이용 가능한 저온 연속 암 조건에서의 저장 중 오이 접목묘의 품질 변화를 구명하였다. 제 1 장에서는 기온 6, 9, 12, 15 및 18°C 로 설정한 저장고에서 연속 암 조건으로 15 일간 저장하며 묘 품질과 이들을 정식한 후의 생존율 및 초기 생육을 조사한 결과, 저장 기온이 높을수록 도장 정도와 엽록소 함량 감소의 폭이 컸으며 생존율이 낮았다. 각각 3, 6, 9, 12, 15 일로 기간을 설정하여 저장한 후 저장묘를 온실에 정식하고 15 일간 재배한 결과, 3 일간 저장한 오이 접목묘는 저장 기온에 관계없이 외관상 묘소질 저하 정도가 미미하였고 정식 후 생존율과 초기 생육은 저장하지 않은 묘와 차이가 없었다. 그러나 저장 기간이 길수록 묘소질 저하 및 생육 저해가 심화되었다. 제 2 장에서는 6, 10, 14, 18°C 의 일정한 기온과, 제 1 장의 실험 결과를 바탕으로 3 일간 저장 시 저장 결과가 가장 좋았던 6°C 조건에서 3 일간 저장 후 저장 기온을 다양한 패턴으로 변화시키면서 묘소질의 변화와 엽록소 함량 및 체내 유리당의 감소를 조사하였다. 6°C 에서 3 일간, 그 이후 저장 기온을 18°C 로 변경한 처리구를 제외한 모든 처리구에서 저장 중 도장을 억제할 수 있었다. 또한 저장 중의 초장 증가와 엽록소 감소 정도뿐 아니라 저장성에 영향을 미치는 식물체 내 유리당 소모 정도 역시 저장 중의 적산 기온과 비례하여 증가하는 것을 확인하였다. 저장 중의 환경적 스트레스를 평가하기 위하여 지질과산화 정도와 DPPH 래디컬 소거능 활성을 측정하였으나 기온 패턴 처리에 따른 일정한 경향을 발견하기 어려웠다. 공정묘 저장기술 개발의 초기 연구로서 국내 육묘 산업의 주요 품목인 오이 접목묘를

대상으로 수행한 본 연구를 통해 저온 약광 저장 기술의 주요 개념 및 전략 설정에 공헌할 수 있는 중요한 결과를 제시할 수 있었다.